PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 2307K-1597PC	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2005/005263	International filing date *(day/month/year)* 17 February 2005 (17.02.2005)	Priority date *(day/month/year)* 20 February 2004 (20.02.2004)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant THE REGENTS OF THE UNIVERSITY OF C ALIFORNIA		

This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

This REPORT consists of a total of 7 sheets, including this cover sheet.

In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

This report contains indications relating to the following items:

- ☒ Box No. I — Basis of the report
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 22 August 2006 (22.08.2006)
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Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Applicant's or agent's file reference 2307K-1597PC	Date of mailing (*day/month/year*) 06 APR 2006 **FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US05/05263	17 February 2005 (17.02.2005)	20 February 2004 (20.02.2004)

International Patent Classification (IPC) or both national classification and IPC

IPC: **C12Q 1/68**(2006.01);**CO7H 21/04**
USPC: 435/6;536/24.33,24.31

Applicant

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☐ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201		David C. Thomas Telephone No. 571-272-3320

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US05/05263

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US05/05263

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 5-6, 11-13, 15-23, 25-27	YES
	Claims 1-4, 7-10, 14, 24	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-27	NO
Industrial applicability (IA)	Claims 1-27	YES
	Claims NONE	NO

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:
Claims 1-4, 7-10, 14 and 24 lack novelty under PCT Article 33(2) as being anticipated by Kopreski et al (U.S. Patent No. 6,607,898).

Kopreski teaches a method of claims 1 and 2 to detect an extracellular mRNA in a bodily fluid, the bodily fluid including a cell phase and a fluid phase (see abstract), the method comprising: providing a cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38); and detecting the extracellular mRNA in the cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38 and 58-60), wherein the bodily fluid is saliva (column 2, line 33).
With regard to claim 3, Kopreski teaches detecting the extracellular mRNA comprising isolating the extracellular mRNA from the cell-free fluid phase portion of the bodily fluid and amplifying the extracellular mRNA (column 2, lines 30-38 and 58-60 and column 5, lines 12-23).
With regard to claim 4, Kopreski teaches performing transcriptome analysis of saliva (column 4, lines 38-52).
With regard to claim 7, Kopreski teaches detecting a transcriptome pattern by quantitative PCR analysis (column 4, lines 38-52 and column 7, lines 46-58).
With regard to claims 8 and 9, Kopreski teaches detecting genetic alterations in a gene in an organ by analyzing a bodily fluid draining from the organ (column 4, lines 52-57), detecting a transcriptome pattern or mRNA profile in the cell-free fluid phase portion (column 4, lines 38-52) and comparing the transcriptome pattern or mRNA profile with a predetermined normal pattern (column 8, lines 18-19), the bodily fluid being cell-free saliva (column 4, line 54).
With regard to claims 10, 14, and 24, Kopreski teaches a method to diagnose an oral or systemic pathology, disease or disorder in a subject (column 7, lines 1-14) by detecting a trascriptome pattern or an mRNA profile in cell-free saliva associated with the pathology, disease or disorder (column 4, lines 38-52 and 54) and comparing the trascriptome pattern or mRNA profile with a predetermined trascriptome pattern or mRNA profile which is indicative of the presence of the pathology, disease or disorder in the subject (column 8, lines 18-19).

Claims 1-3 lack novelty under PCT Article 33(2) as being anticipated by Gocke et al (U.S. Patent No. 6,511,805).

Gocke teaches a method of claims 1 and 2 to detect an extracellular mRNA in a bodily fluid, the bodily fluid including a cell phase and a fluid phase, the method comprising: providing a cell-free fluid phase portion of the bodily fluid (column 5, lines 4-9); and detecting the extracellular mRNA in the cell-free fluid phase portion of the bodily fluid (column 5, lines 13-18 and column 9, lines 9-22), wherein the bodily fluid is saliva (column 5, line 7).
With regard to claim 3, Gocke teaches detecting the extracellular mRNA comprising isolating the extracellular mRNA from the cell-free

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fluid phase portion of the bodily fluid (column 5, lines 13-18) and amplifying the extracellular mRNA (column 8, line 67 to column 9, line 22).

Claims 5 and 6 lack an inventive step under PCT Article 33(3) as being obvious over Kopreski et al (U.S. Patent No. 6,607,898) in view of Beals (U.S. Patent No. 6,617,112).

Kopreski teaches a method of claims 1 and 2 to detect an extracellular mRNA in a bodily fluid, the bodily fluid including a cell phase and a fluid phase (see abstract), the method comprising: providing a cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38); and detecting the extracellular mRNA in the cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38 and 58-60), wherein the bodily fluid is saliva (column 2, line 33) and also teaches isolating the extracellular mRNA from the cell-free fluid phase portion of the bodily fluid and amplifying the extracellular mRNA (column 2, lines 30-38 and 58-60 and column 5, lines 12-23). Kopreski also teaches a method to perform transcriptome analysis by quantitative PCR analysis (column 4, lines 38-52 and column 7, lines 46-58).

Kopreski does not teach a method to perform transcriptome analysis using a high-density oligonucleotide microarray assay.

With regard to claims 5 and 6, Beals teaches a method wherein detecting a transcriptome pattern is performed by a high-density oligonucleotide microarray assay (column 3, lines 21-30 and column 5, lines 12-18).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to combine the methods of Kopreski and Beals, since Kopreski provides a method to detect extracellular mRNA in saliva, while Beals provides a method to analyze and measure transcription of a large number of genes that may be important in diagnosis of various disorders, especially cancers that shed nucleic acids into bodily fluids, including extracellular mRNA.

Claims 11, 15, 17, 19, 21, and 25 lack an inventive step under PCT Article 33(3) as being obvious over Kopreski et al (U.S. Patent No. 6,607,898) in view of Huang et al., (Cancer Res. (1999) 59: 1599-1605).

Kopreski teaches a method of claims 1 and 2 to detect an extracellular mRNA in a bodily fluid, the bodily fluid including a cell phase and a fluid phase (see abstract), the method comprising: providing a cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38); and detecting the extracellular mRNA in the cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38 and 58-60), wherein the bodily fluid is saliva (column 2, line 33) and also teaches isolating the extracellular mRNA from the cell-free fluid phase portion of the bodily fluid and amplifying the extracellular mRNA (column 2, lines 30-38 and 58-60 and column 5, lines 12-23). Kopreski also teaches a method to perform transcriptome analysis by quantitative PCR analysis (column 4, lines 38-52 and column 7, lines 46-58). Kopreski also teaches a method to diagnose an oral or systemic pathology, disease or disorder in a subject (column 7, lines 1-14) by detecting a trascriptome pattern or an mRNA profile in cell-free saliva associated with the pathology, disease or disorder (column 4, lines 38-52 and 54) and comparing the trascriptome pattern or mRNA profile with a predetermined trascriptome pattern or mRNA profile which is indicative of the presence of the pathology, disease or disorder in the subject (column 8, lines 18-19).

Kopreski does not teach methods to diagnose an oral or systemic pathology, disease or disorder in a subject by comparing RNA profiles, wherein the gene is IL-1B or IL6, or a kit containing an identifier and detector for the biomarkers IL-1B or IL6.

With regard to claims 11, 15, 17, 19, and 25, Huang teaches a method to diagnose an oral or systemic pathology, disease or disorder in a subject, including a cancer (nasopharyngeal carcinoma, see abstract), by comparing RNA profiles (Table 3), wherein the disease is a cancer of the oral cavity and the gene is IL-1B or IL6 (p. 1600, lines 9-18 and Table 3).
With regard to claim 21, Huang also provides a kit containing identifiers (primers in Table 2) of biomarkers (IL1B and IL6, Table 2 and p. 1600, column 1, lines 9-11) for an oral or systemic pathology, disease or disorder and also detectors used to detect the identifier (probes in Table 2).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to combine the methods of Kopreski and Huang, since Kopreski provides a method to detect extracellular mRNA in saliva, while Huang provides a method to analyze and measure transcription of a large number of cytokine genes such as IL1B and IL6 that may be important in diagnosis of various disorders, especially cancers that shed nucleic acids into bodily fluids, including extracellular mRNA.

Claims 12, 13, 16, 18, and 20 lack an inventive step under PCT Article 33(3) as being obvious over Kopreski et al (U.S. Patent No. 6,607,898) in view of Chen et al., (Clinical Cancer Res. (1999) 5: 1369-1379).

Kopreski teaches a method of claims 1 and 2 to detect an extracellular mRNA in a bodily fluid, the bodily fluid including a cell phase and a fluid phase (see abstract), the method comprising: providing a cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38); and detecting the extracellular mRNA in the cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38 and 58-60), wherein the bodily fluid is saliva (column 2, line 33) and also teaches isolating the extracellular mRNA from the cell-free fluid phase portion of the bodily fluid and amplifying the extracellular mRNA (column 2, lines 30-38 and 58-60 and column 5, lines 12-23). Kopreski also teaches a method to perform transcriptome analysis by quantitative PCR analysis (column 4, lines 38-52 and column 7, lines 46-58). Kopreski also teaches a method to diagnose an oral or systemic pathology, disease or disorder in a subject (column 7, lines

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In case the space in any of the preceding boxes is not sufficient.

1-14) by detecting a trascriptome pattern or an mRNA profile in cell-free saliva associated with the pathology, disease or disorder (column 4, lines 38-52 and 54) and comparing the trascriptome pattern or mRNA profile with a predetermined trascriptome pattern or mRNA profile which is indicative of the presence of the pathology, disease or disorder in the subject (column 8, lines 18-19).

Kopreski does not teach methods to diagnose an oral or systemic pathology, disease or disorder in a subject, including oropharyngeal squamous cell carcinoma or head and neck squamous cell carcinoma, by comparing RNA profiles, wherein the gene is IL8 or IL6.

With regard to claims 12, 13, 16, 18, and 20, Chen teaches a cancer of the oral cavity, including head and neck squamous cell carcinoma (HNSCC, see abstract), and expression of the genes coding for IL8 and IL6, including the protein profile of the biomarker IL6 (p. 1370, column 1, lines 11-19, p. 1372, lines 23-35, and Table 4).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to combine the methods of Kopreski and Chen, since Kopreski provides a method to detect extracellular mRNA in saliva, while Chen provides a method to analyze and measure expression of a large number of cytokine genes such as IL8 and IL6 that may be important in diagnosis of various disorders, especially head and neck squamous cell carcinomas that shed nucleic acids into bodily fluids such as saliva, including extracellular mRNA and proteins. Though Chen provides a method to measure only protein expression levels, it would be obvious to modify the method to include measurement of mRNA levels as taught by Kopreski to provide a transcriptome.

Claims 22, 23, 26, and 27 lack an inventive step under PCT Article 33(3) as being obvious over Kopreski et al (U.S. Patent No. 6,607,898) in view of Huang et al., (Cancer Res. (1999) 59: 1599-1605) and further in view of Chen et al., (Clinical Cancer Res. (1999) 5: 1369-1379).

Kopreski teaches a method of claims 1 and 2 to detect an extracellular mRNA in a bodily fluid, the bodily fluid including a cell phase and a fluid phase (see abstract), the method comprising: providing a cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38); and detecting the extracellular mRNA in the cell-free fluid phase portion of the bodily fluid (column 2, lines 30-38 and 58-60), wherein the bodily fluid is saliva (column 2, line 33) and also teaches isolating the extracellular mRNA from the cell-free fluid phase portion of the bodily fluid and amplifying the extracellular mRNA (column 2, lines 30-38 and 58-60 and column 5, lines 12-23). Kopreski also teaches a method to perform transcriptome analysis by quantitative PCR analysis (column 4, lines 38-52 and column 7, lines 46-58). Kopreski also teaches a method to diagnose an oral or systemic pathology, disease or disorder in a subject (column 7, lines 1-14) by detecting a trascriptome pattern or an mRNA profile in cell-free saliva associated with the pathology, disease or disorder (column 4, lines 38-52 and 54) and comparing the trascriptome pattern or mRNA profile with a predetermined trascriptome pattern or mRNA profile which is indicative of the presence of the pathology, disease or disorder in the subject (column 8, lines 18-19).

Kopreski does not teach methods to diagnose an oral or systemic pathology, disease or disorder in a subject, including oropharyngeal squamous cell carcinoma or head and neck squamous cell carcinoma, by comparing RNA profiles, wherein the gene is IL8, IL1B, or IL6.

Huang teaches a method to diagnose an oral or systemic pathology, disease or disorder in a subject, including a cancer (nasopharyngeal carcinoma, see abstract), by comparing RNA profiles (Table 3), wherein the disease is a cancer of the oral cavity and the gene is IL-1B or IL6 (p. 1600, lines 9-18 and Table 3). Huang also provides a kit containing identifiers (primers in Table 2) of biomarkers (IL1B and IL6, Table 2 and p. 1600, column 1, lines 9-11) for an oral or systemic pathology, disease or disorder and also detectors used to detect the identifier (probes in Table 2).

Huang does not teach methods to diagnose an oral or systemic pathology, disease or disorder in a subject, including oropharyngeal squamous cell carcinoma or head and neck squamous cell carcinoma.

With regard to claims 22, 23, 26, and 27, Chen teaches a cancer of the oral cavity, including head and neck squamous cell carcinoma (HNSCC, see abstract), and expression of the genes coding for IL8 and IL6, including the protein profile of the biomarker IL6 (p. 1370, column 1, lines 11-19, p. 1372, lines 23-35, and Table 4).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to combine the methods of Kopreski, Huang, and Chen, since Kopreski provides a method to detect extracellular mRNA in saliva, Huang teaches methods of RNA profiling, including IL1B and IL6, for diagnosing nasopharyngeal carcinoma, while Chen provides a method to analyze and measure expression of a large number of cytokine genes such as IL8 and IL6 that may be important in diagnosis of various disorders, especially head and neck squamous cell carcinomas that shed nucleic acids into bodily fluids such as saliva, including extracellular mRNA and proteins. Though Chen provides a method to measure only protein expression levels, it would be obvious to modify the method to include measurement of mRNA levels as taught by Kopreski and Huang to provide a transcriptome.

Claims 1-27 meet the criteria set out in PCR Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.